FOODBORNE PATHOGENS AND DISEASE
Volume 8, Number 5, 2011

DOI: 10.1089/fpd.2010.0702

Characterization of *Salmonella enterica* Isolates from Turkeys in Commercial Processing Plants for Resistance to Antibiotics, Disinfectants, and a Growth Promoter

Ross C. Beier,[1] Phelue N. Anderson,[2,3] Michael E. Hume,[1] Toni L. Poole,[1] Sara E. Duke,[1] Tawni L. Crippen,[1] Cynthia L. Sheffield,[1] David J. Caldwell,[2] James A. Byrd,[1] Robin C. Anderson,[1] and David J. Nisbet[1]

Abstract

Salmonella enterica isolates from turkeys in two commercial processing plants (1 and 2) were characterized for susceptibility to antibiotics, disinfectants, and the organoarsenical growth promoter, 4-hydroxy-3-nitrophenylarsonic acid (3-NHPAA, roxarsone), and it's metabolites, $NaAsO_2$ (As(III)) and $Na_2HAsO_4 \bullet 7H_2O$ (As(V)). The 130 *Salmonella* serovars tested demonstrated a low incidence of resistance to the antibiotics gentamicin (GEN), kanamycin (KAN), sulfamethoxazole (SMX), streptomycin (STR), and tetracycline (TET). Isolates resistant to antibiotics were most often multidrug resistant. Serovars Hadar and Typhimurium were resistant to KAN, STR, and TET and GEN, SMX, and STR, respectively. All isolated *Salmonella* serovars were resistant to the disinfectant chlorhexidine with minimum inhibitory concentrations (MICs; 1–8 μg/mL), and they were susceptible to triclosan and benzalkonium chloride. The didecyldimethylammonium chloride component was the most active ammonium chloride tested. No cross-resistance was observed between antibiotics and disinfectants. The MICs for 3-NHPAA (4096 μg/mL) were consistent between processing Plant 1 and Plant 2, but MICs for the 3-NHPAA metabolites (As(III) and As(V)) were higher in Plant 1 than in Plant 2. In Plant 1, 76% of the isolates had MICs >256 μg/mL for As(III) and 92% of the isolates had MICs >1024 μg/mL for As(V). In Plant 2, all of the isolates had MICs ≤ 256 μg/mL for As(III) and 90% of the isolates had MICs ≤ 1024 μg/mL for As(V). Only 4 *Salmonella* serovars were isolated from Plant 1, but 10 serovars were isolated from Plant 2. *S. enterica* serovar Derby from Plant 1 was highly resistant to As(III) and As(V) with MICs >1024 and >8192 μg/mL, respectively, suggesting previous exposure to high arsenic metabolite concentrations. These levels may have been high enough to kill other *Salmonella* serovars, thus possibly explaining the lack of serovar diversity observed in Plant 1. The application of a growth promoter may affect the serovar diversity in treated birds.

Introduction

Salmonella enterica infections from foodborne sources are significant threats to human health worldwide, as well as within the United States (Jørgensen *et al.*, 2002; Liljebjelke *et al.*, 2005), and infections can cause serious illnesses or fatalities in elderly and immunocompromised humans. The Centers for Disease Control and Prevention estimated that each year in the United States over 1.3 million human illnesses, over 15,000 hospitalizations, and 553 deaths are caused by foodborne transmission of *Salmonella* (Mead *et al.*, 1999). *Salmonella* was estimated to cause more deaths in the United States from foodborne illnesses than any other known pathogen (Mead *et al.*, 1999), and was estimated to be the leading cause of bacterial pathogen foodborne illness in the United States (CDC, 2007). The Economic Research Service estimated that the cost of medical treatment, time lost from work, and premature death from all sources of *Salmonella* infections in 2008 dollars was over $2.6 billion per year (USDA-ERS, 2010).

Poultry meat and eggs are considered to be the major vehicles for transmission of *Salmonella* to humans, and *Salmonella* has been frequently reported in products of meat and poultry processing plants (Li and Mustapha, 2002; Capita *et al.*, 2003; Vadhanasin *et al.*, 2004). The processing plant is considered one of several routes causing contamination of poultry (Corry *et al.*, 2002). Disinfectants or biocides are chemical agents that inhibit or kill a broad-spectrum of

[1]Southern Plains Agricultural Research Center, Agricultural Research Service, U.S. Department of Agriculture, College Station, Texas.
[2]Department of Poultry Science, Texas A&M University, College Station, Texas.
[3]Texas Veterinary Medical Diagnostic Laboratory, Poultry Diagnostic Laboratory, Center, Texas.

microorganisms (White and McDermott, 2001), and their use for sanitation and bacteria control in the home, medicine, agricultural production, and processing plants is crucial to protecting the food supply and decreasing the risk of human exposure to pathogens (Beier *et al.*, 2004).

Reports suggest that reduced bacterial susceptibility to biocides is increasing (McDonnell and Russell, 1999; Russell, 2002), and others describe increased bacterial resistance to disinfectants in areas where they have been used (Mølbak *et al.*, 1999; Ferber, 2000). Many organisms possess multidrug efflux systems capable of exporting a wide-range of compounds (Nikaido, 1996; Levy, 2002). Biocide use may result in the development of cross-resistance to antibiotics (Maris, 1991; Sidhu *et al.*, 2002a; Braoudaki and Hilton, 2004). Exposure to only a single drug may lead to cross-resistance with many structurally and functionally unrelated drugs (George, 1996). Sidhu *et al.* (2002a) indicated a link between biocide and antibiotic resistance, and we demonstrated cross-resistance in beta-hemolytic *Escherichia coli* with reduced susceptibility to chlorhexidine and resistance to gentamicin and streptomycin (Beier *et al.*, 2005). However, the reports describing a link between disinfectants and antibiotics are limited. Cross-resistance between antibiotics and disinfectants could potentially pose a great challenge in the control of pathogens and the use of disinfectants.

Little is known of the disinfectant resistance profiles of pathogens in settings where there is widespread use of disinfectants, such as in poultry processing plants. The aim of this study was to describe the distribution of minimum inhibitory concentrations (MICs) for antibiotics, disinfectants, and the growth promoter 4-hydroxy-3-nitrophenylarsonic acid (3-NHPAA, roxarzone) and its metabolites $NaAsO_2$ (As(III)) and $Na_2HAsO_4 \bullet 7H_2O$ (As(V)) (both are known antimicrobials) (Sapkota *et al.*, 2006) in *Salmonella* serovars isolated from turkeys in commercial plants. This evaluation will also determine the effects of the arsenic compounds on these bacterial isolates, and whether these isolates demonstrate a link between antibiotic resistance and disinfectant susceptibility.

Materials and Methods

Bacterial serovars

One-hundred thirty *Salmonella* isolates used in this study were obtained from two commercial turkey processing facilities (Plant 1 and Plant 2), located in different geographical regions of the United States. *Salmonella* isolation procedures were similar to those used for *Campylobacter* (Caldwell *et al.*, 2003; Byrd *et al.*, 2006). Briefly, on each day of sampling (2 days in 2002 and 1 day in 2004), ~100 carcass rinse samples were obtained from both pre- and postimmersion chiller sampling sites for determination of *Salmonella*. The rinse fluid was placed into sterilized polypropylene collection bottles. All sample collection bottles were placed on wet ice and transported back to our laboratories to be cultured. This procedure resulted in ~200 samples per sampling day per facility for a total of 600 samples from each of the two facilities or 1200 samples in total. Plant 1 had 122/600 (20.3%) samples positive for *Salmonella*, and 153/600 (25.5%) samples from plant 2 were positive for *Salmonella* (Anderson *et al.*, 2010). Four *Salmonella* serovars were obtained from plant 1, Derby (101), Hadar (9), Montevideo (4), and Senftenberg (8), and 10 serovars were obtained from plant 2, Agona (9), Anatum (1), Brandenburg (29), Derby (21), Hadar (17), Meleagridis (3), Montevideo (4), Reading (8), Senftenberg (2), and Typhimurium (59) (Anderson *et al.*, 2010). After isolation, all isolates were stored at −80°C in tryptic soy broth containing 20% (v/v) glycerol until needed in this investigation. A randomly selected group of *Salmonella* isolates from plants 1 (25) and 2 (105) was used in this work and comprised all serovars found in the two plants.

Antimicrobial susceptibility testing

MICs were determined by broth microdilution according to the Clinical and Laboratory Standards Institute (CLSI, 2005, 2006). MICs were determined as the lowest concentration of compound that inhibited the visible growth of the microorganisms (Andrews, 2001). Antibiotic MICs were obtained using the National Antimicrobial Resistance Monitoring System Sensititre® Gram-negative plate (CMV1AGNF) and the Sensititre fluoroquinolone plate (CMV1DW) during 2006, demineralized water (5 mL), and cation-adjusted Mueller-Hinton broth with TES (Tris, EDTA, and NaCl, pH 8) buffer (11 mL) were obtained from Trek Diagnostic Systems Inc. (Cleveland, OH), and MICs of the 16 antimicrobials (Table 1) were determined using the Sensititre automated susceptibility system according to the manufacturer's instructions (TDSI). *Pseudomonas aeruginosa* 27853 and *E. coli* 25922 were used as controls for antibiotic susceptibility testing, and *E. coli* 25922 was the control for disinfectant susceptibility testing.

Disinfectant and growth promoter susceptibility testing

The disinfectants and disinfectant components used in the study along with the recommendations for where they should be used as well as where they were obtained are listed in Table 2. The growth promoter, 3-NHPAA, was obtained from Acros Organics (Morris Plains, NJ), and the 3-NHPAA metabolite, As(III), was obtained from Alfa Aesar (Ward Hill, MA). The 3-NHPAA metabolite, As(V), and sterile dimethyl sulfoxide (used to solubilize some disinfectants) were obtained from Sigma-Aldrich (St. Louis, MO). Reverse osmosis water (H_2O) was produced on site by a reverse osmosis system obtained from Millipore Corp. (Bedford, MA). The following disinfectants exist as mixtures of multiple components: DC&R® has the following active ingredients: (THN, 19.2%; (C12BAC-67%, C14BAC-25%, C16BAC-7%, and (C8, C10, C18)-1%)benzyldimethylammonium chlorides), 3.08%; and formaldehyde, 2.28%. The active ingredients of Enforcer® are the following: (C12BAC-5%, C14BAC-60%, C16BAC-30%, and C18 benzyldimethylammonium chloride-5%), 0.105%, and (C12BAC-68%, C14BAC-32%), 0.105%. The active ingredients of Tek-Trol® are *o*-Phenylphenol, 12%; *o*-benzyl-*p*-chlorophenol, 10%; and *p*-tert-amylphenol, 4%; and the active ingredients of P-128 are C10AC, 4.61%, and (C12BAC-40%, C14BAC-50%, and C16BAC-10%), 3.07%. The MICs for these disinfectants were determined on the composite mixtures.

All chemicals were diluted with H_2O and filter sterilized using a 0.2 μm×25 mm syringe filter (No. 431224; Corning Inc., Corning, NY), and dimethyl sulfoxide (≤5%) was also added to triclosan, C14BAC, C16BAC, THN, and 3-NHPAA to aid chemical solubility, and the final solutions contained Mueller Hinton broth (DIFCO brand Mueller Hinton Broth, No. 275730; Fisher Scientific, Houston, TX). The following

Table 1. National Antimicrobial Resistance Monitoring System Overall Antibiotic Minimum Inhibitory Concentrations and Resistance Profiles Among *Salmonella enterica* Isolated from Turkeys[a]

Antibiotic	*MIC_{50} (μg/mL)*	*MIC_{90} (μg/mL)*	*Range (μg/mL)*	*No. (%) Resistant*	*Break point*
AMI	1	2	≤0.5–5	0 (0)	≥64
AMP	≤1	2	≤1–>32	1 (0.8)	≥32
AUG	≤1/0.5	≤1/0.5	≤1/0.5–16/8	0 (0)	≥32/16
AXO	≤0.25	≤0.25	≤0.25–0.5	0 (0)	≥64
CEP	4	8	≤2–16	0 (0)	≥32
CHL	8	8	4–8	0 (0)	≥32
CIP	0.03	0.03	≤0.015–0.06	0 (0)	≥4
COT	≤0.12/2.38	≤0.12/2.38	≤0.12/2.38–≥4/76	2 (1.5)	≥4/76
FOX	4	8	2–16	0 (0)	≥32
GEN	≤0.25	16	≤0.25–>16	29 (22)	≥16
KAN	8	8	≤8–>64	10 (8)	≥64
NAL	4	8	1–16	0 (0)	≥32
SMX	64	>512	≤16–>512	49 (38)	≥512
STR	≤32	64	≤32–>64	42 (32)	≥64
TET	≤4	>32	≤4–>32	17 (13)	≥16
TIO	1	1	≤0.12–8	1 (0.8)	≥8

[a]NARMS Gram-negative antibiotic overall MIC profiles of 130 *Salmonella enterica* isolates from turkeys in two commercial processing plants.

AMI, Amikacin; AMP, Ampicillin; AUG, Amoxicillin/Clavulanic acid; AXO, Ceftriazone; CEP, Cephalothin; CHL, Chloramphenicol; CIP, Ciprofloxacin; COT, Trimethoprim/Sulfamethoxazole; FOX, Cefoxitin; GEN, Gentamicin; KAN, Kanamycin; NAL, Nalidixic acid; SMX, Sulfamethoxazole; STR, Streptomycin; TET, Tetracycline; TIO, Ceftiofur; MIC, minimum inhibitory concentration; NARMS, National Antimicrobial Resistance Monitoring System.

disinfectant concentrations were tested for susceptibility as previously described (Beier *et al.*, 2005, 2008): DC&R, 1024–1 μg/mL; Tek-Trol, 512–0.5 μg/mL; chlorhexidine, 64–0.06 μg/mL; triclosan, 4–0.004 μg/mL; Enforcer, 64–0.06 μg/mL; P-128, 64–0.06 μg/mL; BKC, 256–0.25 μg/mL; P-I, 32768–32 μg/mL; formaldehyde, 2048–2 μg/mL; THN, 4096–4 μg/mL; C10AC, 64–0.06 μg/mL; C12BAC, 512–0.5 μg/mL; C14BAC, 128–0.12 μg/mL; C16BAC, 128–0.12 μg/mL; 3-NHPAA, 16384–16 μg/mL; As(III), 1024–1 μg/mL; and As(V), 8192–8 μg/mL.

Statistical analysis

Statistical analysis was conducted in JMP 6.0©, SAS Institute Inc. (Cary, NC). Data were analyzed using multivariate hierarchical clustering techniques and Ward's minimum variance method to produce dendrograms that could demonstrate relationships between MICs. Presumptive associations among isolates were evaluated through correlation analysis. Kendall's tau nonparametric correlation coefficient was used since the variables were not continuous. The test for correlations were assessed at a 0.05 probability of Type I error.

Results

Antimicrobial susceptibility characteristics

Resistance profiles of the 130 *Salmonella* isolates for the National Antimicrobial Resistance Monitoring System Gram-negative antibiotic panel are shown in Table 1. The *Salmonella* isolates demonstrated the highest percentage of resistance to SMX, at 38%, followed by STR, GEN, TET, and KAN at a resistance percentage of 32%, 22%, 13%, and 8%, respectively. All *Salmonella* isolates tested were susceptible (data not shown) to the fluoroquinolone antibiotics, CIP, DANO, DIF,

Table 2. Disinfectants and Disinfectant Components

Disinfectants	*Abbreviation*
Benzalkonium chloride[a–c]	BKC
Chlorhexidine diacetate (Nolvasan® solution)[a,b,d,e]	Chlorhexidine
DC&R[a,d,e]	DC&R
Enforcer[a,b,f,g]	Enforcer
J.T. Baker 37% formaldehyde solution[h]	Formaldehyde
P-128[a,b,d,i,j]	P-128
Betadine® solution, 10% providone-iodine[a,b,k]	P-I
Tek-Trol[a,d,e]	Tek-Trol
Irgasan[b,c,f]	Triclosan
Disinfectant components	
Didecyldimethylammonium chloride[l]	C10AC
Benzyldimethyldodecylammonium chloride[m]	C12BAC
Benzyldimethyltetradecylammonium chloride[c]	C14BAC
Benzyldimethylhexadecylammonium chloride[c]	C16BAC
Tris(hydroxylmethyl)nitromethane[c]	THN

[a]Recommended for use in Veterinary clinics.
[b]Recommended for use in hospitals.
[c]Obtained from Sigma-Aldrich (Milwaukee, WI).
[d]Recommended for use on the farm.
[e]Obtained from the Producers Cooperative Association (Bryan, TX).
[f]Recommended for use in the home.
[g]Recommended for use in restaurants.
[h]Obtained from VWR International, Inc. (Marietta, GA).
[i]Recommended for use in federally inspected meat and poultry establishments.
[j]Obtained from Burns Veterinary Supply, Inc. (Farmers Branch, TX).
[k]Obtained from The Pharmacy Shop (Bryan, TX).
[l]Obtained from Lonza Inc. (Fairlawn, NJ).
[m]Obtained from Fluka Analytical (Sigma-Aldrich, St. Louis, MO).

Table 3. Distribution of Disinfectant and Disinfectant Component Susceptibility Profiles Among *Salmonella enterica* Isolated from Turkeys[a]

	MIC (μg/mL)																		
Disinfectant[b]	*0.12*	*0.25*	*0.5*	*1*	*2*	*4*	*8*	*16*	*32*	*64*	*128*	*256*	*512*	*1024*	*2048*	*4096*	*8192*	MIC_{50} (μg/mL)	MIC_{90} (μg/mL)
DC&R										1[c]	126	3						128	256
Tek-Trol											20	109	1					256	256
Chlorhexidine				1	24	93	12											4	4
Triclosan	3	46	68	13														0.5	0.5
Enforcer						1	25	104										16	16
P-128						30	99	1										8	8
BKC								2	128									32	32
P-I																28	102	8192	8192
C10AC[d]					7	120	3											4	4
C12BAC[d]								2	128									4	8
C14BAC[d]								105	25									16	32
C16BAC[d]								109	21									16	32
THN[d]												29	90	11				512	512
Formaldehyde[d]								8	112	10								32	32

[a]Overall susceptibility profiles of 130 *S. enterica* isolates from turkeys in two commercial processing plants.
[b]Disinfectant and disinfectant component abbreviations: benzalkonium chloride (BKC), providone-iodine (P-I), didecyldimethylammonium chloride (C10AC), benzyldimethyldodecylammonium chloride (C12BAC), benzyldimethyltetradecylammonium chloride (C14BAC), benzyldimethylhexadecylammonium chloride (C16BAC), and tris(hydroxylmethyl)nitromethane (THN).
[c]Indicates the total number of isolates out of 130 total *Salmonella* that exhibited the indicated MIC.
[d]These entries are disinfectant components.

ENRO, GAT, LEVO, MARB, and ORB. DIF had the highest MIC_{90} value of 0.5 μg/mL.

Disinfectant susceptibility characteristics

Table 3 shows the MIC profiles of the 130 Salmonella serovars for the disinfectants and disinfectant components tested. The isolates were susceptible to triclosan, and demonstrated the highest MICs, 4096 and 8192 μg/mL, to P-I. The chlorhexidine MICs were within the range of 1–8 μg/mL. The Salmonella isolates were less susceptible to the benzylammonium chlorides, C12BAC, C14BAC, and C16BAC (at 16 and 32 μg/mL), than they were to the non-benzylammonium chloride C10AC (2–8 μg/mL). The bacteria demonstrated BKC MICs of 16 and 32 μg/mL, and had high levels of susceptibility to the disinfectant component

Table 4. Distribution of As(III) Susceptibility Profiles Among *Salmonella enterica* Serovars Isolated from Turkeys

	MIC (μg/mL)													
Plant 1[a] *Serovar*	*16*	*32*	*64*	*128*	*256*	*512*	*1024*	*>1024*	*2048*	*4096*	*8192*	*>8192*	MIC_{50} (μg/mL)	MIC_{90} (μg/mL)
Salmonella Derby		1					13	6					1024	>1024
Salmonella Hadar		1											[b]	32
Salmonella Montevideo				2									128	128
Salmonella Senftenberg				2									128	128
Plant 2[c] *Serovar*														
Salmonella Agona		2	1	1									32	128
Salmonella Anatum					1								[b]	256
Salmonella Brandenburg		21											32	32
Salmonella Derby		13	2	1									32	64
Salmonella Hadar	1	12		1									32	32
Salmonella Meleagridis				2									128	128
Salmonella Montevideo		1	1	2									64	128
Salmonella Reading		3	5										64	64
Salmonella Senftenberg				2									128	128
Salmonella Typhimurium		33											32	32

[a]There were 25 randomly selected *Salmonella* isolates from Plant 1.
[b]There was only one isolate in this group.
[c]There were 105 randomly selected *Salmonella* isolates from Plant 2.

THN with MICs ranging from 256–1024 μg/mL. These bacteria also demonstrated high levels of susceptibility to the two disinfectants, DC&R and Tek-Trol, at 64–256 μg/mL and 128–512 μg/mL, respectively, and the susceptibility levels for both Enforcer and P-128 were from 4–16 μg/mL, although most Enforcer MICs were at the high end of the range.

Salmonella isolates (24/25, 96%) in Plant 1 had chlorhexidine MICs of 4 or 8 μg/mL, and the 25th isolate (4%) had a chlorhexidine MIC of 2 μg/mL. In Plant 2, *Salmonella* isolates (81/105, 77%) had chlorhexidine MICs of 4 or 8 μg/mL.

Growth promoter susceptibility characteristics

Salmonella 3-NHPAA MICs were at the high levels of 2048 or 4096 μg/mL in both Plants (data not shown). Table 4 shows the breakdown of the 3-NHPAA metabolite As(III) susceptibility profiles among the *Salmonella* serovars in Plants 1 and 2. The As(III) MICs ranged from 16 to >1024 μg/mL. Plant 1 had 13 isolates with an As(III) MIC of 1024 μg/mL and 6 isolates with an As(III) MIC >1024 μg/mL. Therefore, 76% of the isolates from Plant 1 had an As(III) MIC of 1024 μg/mL or greater, whereas only ~1% of the isolates from Plant 2 had an As(III) MIC >128 μg/mL.

Table 5 shows the breakdown of the 3-NHPAA metabolite As(V) susceptibility profiles among the *Salmonella* serovars in Plants 1 and 2. The As(V) MICs ranged from 128 to >8192 μg/mL. Plant 1 had 23/25 (92%) isolates with an As(V) MIC of ≥4096 μg/mL, but only ~6% of the isolates from Plant 2 had an As(V) MIC of 4096 μg/mL.

In Plant 1, 76% of the isolates had As(III) MICs >256 μg/mL and 92% of the isolates had As(V) MICs >1024 μg/mL. In Plant 2, all of the isolates had As(III) MICs ≤256 μg/mL and 90% of the isolates had As(V) MICs ≤1024 μg/mL.

Calculation of DC&R component MICs

An individual component MIC of DC&R can be calculated by multiplying the DC&R MIC by the component of interest percentage and dividing by the sum of all the component percentages in DC&R. For example, to calculate the MIC of the benzyldimethylammonium chloride (BAC) component (primarily C12BAC, C14BAC, and C16BAC) of DC&R, at the DC&R MIC = 128 μg/mL (Table 3), the BAC component level = 128 μg/mL×3.08/24.56 = 16 μg/mL. In similar fashion, the calculated THN portion of DC&R results in a distribution of 50, 100, and 200 μg/mL THN for the DC&R MICs of 64, 128, and 256 μg/mL (Table 3), and the calculated formaldehyde portion of the DC&R MIC results in a distribution of 5.9, 11.88, and 23.76 μg/mL.

Statistical analysis

There were no statistical correlations between the bacterial patterns of susceptibility for the antibiotics and the disinfectants.

Discussion

Antimicrobial susceptibility characteristics

A low percentage of resistance among isolates was observed for five antibiotics, GEN, KAN, SMX, STR, and TET, but these isolates were most often multiresistant. In general, *Salmonella* Hadar was resistant to KAN, STR, and TET, and *Salmonella* Typhimurium was resistant to GEN, SMX, and STR. Braoudaki and Hilton (2004) observed cross-resistance to chlorhexidine when *Salmonella* Typhimurium was adapted to erythromycin, but no such cross-resistance was observed among these wild-type *Salmonella* isolates between the antibiotics and disinfectants tested. We previously observed cross-resistance in beta-hemolytic *E. coli* between chlorhexidine and the antibiotics gentamicin and streptomycin (Beier *et al.*, 2005). The bacteria here tended to have slightly higher MICs for DIF than for the other fluoroquinolone antibiotics. However, all *Salmonella* were susceptible to the fluoroquinolones tested.

Disinfectant susceptibility characteristics

The *Salmonella* isolates were more resistant to the antimicrobial action of DC&R, Tek-Trol, and P-I than they were to chlorhexidine, triclosan, Enforcer, P-128, BKC, and formaldehyde or to several of the individual components, C10AC, C12BAC, C14BAC, and C16BAC. An interesting MIC relationship can be seen when concentrations of individual components are examined and related, as with DC&R, which is a mixture of several components. The calculated levels for the individual components, THN and formaldehyde, in DC&R are well below the levels required for disinfection of *Salmonella* as seen in Table 3. However, the BAC component, comprised of C12BAC, C14BAC, and C16BAC, results in an MIC distribution of 8, 16, and 32 μg/mL, which are equivalent to BAC MICs required for disinfection (Table 3). These BAC concentrations would have accomplished the entire antimicrobial activity of DC&R, and were similar to that obtained with DC&R against vancomycin-resistant *Enterococcus faecium* (VRE) (Beier *et al.*, 2008). It was the BAC portion of DC&R that provided VRE disinfection. The continued use of THN should be reassessed if it demonstrates poor disinfection against other bacteria as it did here and previously against VRE (Beier *et al.*, 2008). However, the application rate of DC&R at 1919 μg/mL is over the level required for killing *Salmonella* (256 μg/mL).

Tek-Trol is a phenolic disinfectant and these isolates demonstrated high-susceptibility to Tek-Trol. However, the *Salmonella* MICs were well below the recommended application rate for Tek-Trol (1016 μg/mL). The continued use of disinfectants such as Tek-Trol and the component THN may function to raise resistance levels in bacteria because of the required high application rates and the poor control obtained, allowing more disinfectant to be present in the environment and a higher chance for adaptive resistance (Braoudaki and Hilton, 2004). It may be appropriate to use chemicals that have much lower bacterial MICs.

The chlorhexidine MIC values were lower here than previously observed for *Salmonella* (2–64 μg/mL) isolated from broilers, cattle, and pig feces (Aarestrup and Hasman, 2004). Chlorhexidine resistance in staphylococci isolates was based on if they could grow at or above a chlorhexidine level of 1 μg/mL (Leelaporn *et al.*, 1994), which is the definition of chlorhexidine resistance used here. The chlorhexidine MICs of *Salmonella* Montevideo (8 μg/mL) in both Plants 1 and 2 were similar to that observed by Block (2001) in clinical isolates. Block (2001) also reported chlorhexidine MICs

Table 5. Distribution of As(V) Susceptibility Profiles Among *Salmonella enterica* Serovars Isolated from Turkeys

Plant 1[a] *Serovar*	*MIC (μg/mL)*											*MIC_{50} (μg/mL)*	*MIC_{90} (μg/mL)*
	16	*32*	*64*	*128*	*256*	*512*	*1024*	*2048*	*4096*	*8192*	*>8192*		
Salmonella Derby							1			15	4	8192	>8192
Salmonella Hadar							1					[b]	1024
Salmonella Montevideo									2			4096	4096
Salmonella Senftenberg									2			4096	4096
Plant 2[c] *Serovar*													
Salmonella Agona							2	2				1024	2048
Salmonella Anatum									1			[b]	4096
Salmonella Brandenburg						16	5					512	1024
Salmonella Derby						3	12	1				1024	2048
Salmonella Hadar				1		4	8	1				1024	2048
Salmonella Meleagridis								2				2048	2048
Salmonella Montevideo						1			3			4096	4096
Salmonella Reading						3	5					1024	1024
Salmonella Senftenberg									2			4096	4096
Salmonella Typhimurium						12	21					1024	1024

[a]There were 25 randomly selected *Salmonella* isolates from Plant 1.
[b]There was only one isolate in this group.
[c]There were 105 randomly selected *Salmonella* isolates from Plant 2.

for *Salmonella* Typhimurium of 8–16 μg/mL; however, we observed *Salmonella* Typhimurium only in Plant 2 with MICs of 2–4 μg/mL. But we also observed high chlorhexidine MICs (8 μg/mL) in Anatum, Brandenburg, and Hadar in Plant 2.

The *Salmonella* triclosan MICs were ≤1, and are susceptible to triclosan. Triclosan acts by inhibiting a highly conserved enzyme enoyl-ACP reductase of bacterial fatty-acid biosynthesis (Heath and Rock, 2000). Braoudaki and Hilton (2004) obtained cross-resistance between antibacterial agents and triclosan in *Salmonella* Typhimurium, but no cross-resistance between antibacterial agents and triclosan was observed here.

Enforcer contains both ethylbenzyl- and BACs and the *Salmonella* Enforcer MICs observed here are not widely different than those of the individual components C14BAC and C16BAC. This is similar to what was observed for VRE (Beier *et al.*, 2008).

One of the P-128 active ingredients (60%) is C10AC. The C10AC component MIC contribution of P-128 was calculated to be a distribution of 2.4, 4.8, and 9.6 μg/mL. This distribution is similar to the MIC distribution of 2, 4, and 8 μg/mL observed for these *Salmonella* by the individual C10AC component. The remaining ingredients displayed higher MICs (16–32 μg/mL). Therefore, C10AC is considered the primary active component in P-128. Further, we determined that C10AC is the most active ammonium chloride tested against these *Salmonella*, as it was against VRE (Beier *et al.*, 2008). C10AC was also the most active ammonium chloride against beta-hemolytic *E. coli* (unpublished).

Salmonella BKC MICs gave a distribution of 16–32 μg/mL, which is the same as observed for C12BAC, C14BAC, and C16BAC. The ability of *Salmonella* Typhimurium to rapidly develop enhanced resistance to BKC has been demonstrated (Joynson *et al.*, 2002; Braoudaki and Hilton, 2004). Sidhu *et al.* (2002b) defined food-associated Gram-negative bacteria susceptible to BKC as having MICs <30 μg/mL BKC, and demonstrated reduced susceptibility to BKC when the MICs were between 30–50 μg/mL BKC. The *Salmonella* evaluated in this study are susceptible to BKC.

The distribution of P-I MICs is 4096 and 8192 μg/mL and are the highest measured MICs of any disinfectant tested. The manufacturer recommends a 100,000 μg/mL solution of P-I to be used directly on surface wounds without dilution. This is about a 12-fold excess over that required for disinfection of *Salmonella*.

Growth promoter susceptibility characteristics

Organoarsenicals are commonly used for growth promotion in U.S. poultry production (Sapkota *et al.*, 2006). 3-NHPAA is used in 70% of the U.S. broiler industry (Sapkota *et al.*, 2006), and is thought to be excreted unchanged in the manure (Garbarino *et al.*, 2003). However, when water was added to the litter, speciation shifted to primarily arsenate (As(V)) (Garbarino *et al.*, 2003). 3-NHPAA and its metabolites, As(III) and As(V), MIC profiles among *Salmonella* are described here. The 3-NHPAA MICs remained consistent between processing Plants 1 and 2. However, the 3-NHPAA metabolites MICs were different in Plant 1 from Plant 2. The results demonstrate that in Plant 1, 76% of the isolates had MICs >256 μg/mL As(III) and 92% of the isolates had MICs >1024 μg/mL As(V). In Plant 2, all of the isolates had MICs ≤256 μg/mL As(III) and 90% of the isolates had MICs ≤1024 μg/mL As(V). This suggests that the birds from Plant 1 may have been exposed to higher quantities of the 3-NHPAA metabolites, As(III) and As(V), than the birds from Plant 2 resulting in adaptive resistance (Braoudaki and Hilton, 2004), or perhaps by accessing integron-linked antibiotic resistance genes (Lapierre *et al.*, 2010). The higher quantities of As(III) and As(V) would have been sufficient to limit the number of *Salmonella* serovars available to be isolated from the birds in Plant 1.

Only four *Salmonella* serovars were isolated from Plant 1, Derby, Hadar, Montevideo, and Senftenberg. An additional six *Salmonella* serovars were isolated from Plant 2, Agona, Anatum, Brandenburg, Meleagridis, Reading, and Typhimurium. *Salmonella* Derby serovars in Plant 1 were highly resistant to As(III) and As(V). With the possibility of the As(III) levels being $>1024\,\mu g/mL$ and the As(V) levels being $>8192\,\mu g/mL$ in Plant 1, these metabolite levels would be high enough to kill other serovars of bacteria, such as those observed in Plant 2 that were not observed in Plant 1. Therefore, we hypothesize that the level of 3-NHPAA metabolites in the turkeys of Plant 1 may have been high enough to kill many of the serovars of *Salmonella* that were observed in Plant 2. The high levels of resistance to the two 3-NHPAA metabolites, As(III) and As(V), observed in *Salmonella* Derby from processing Plant 1 suggests that higher levels of 3-NHPAA may have been provided in the feed, or that a higher rate of production of the metabolites As(III) and As(V) occurred in the turkeys from Plant 1, suggesting that application of a growth promoter may affect serovar diversity in treated birds.

Conclusions

Some *Salmonella* demonstrated a low rate of multidrug resistance (resistance to three or more antibiotics), but all *Salmonella* were susceptible to the 8 fluoroquinolones tested, as well as to triclosan and BKC. These isolates demonstrated chlorhexidine resistance, and didecyldimethylammoniuim chloride (C10AC) was responsible for the disinfection activity of P-128. The C10AC component was the most active ammonium chloride tested against these *Salmonella*. No cross-resistance or link was observed in these *Salmonella* between the antibiotics and disinfectants tested. This is desirable, because cross-resistance between antibiotics and disinfectants would serve to help increase the level of resistance to antibiotics from disinfectant use. Our results demonstrate that susceptibility to disinfectants for many serovars remains well below that of the recommended application rate, and disinfectant use on *Salmonella* should not promote risk of antimicrobial resistance among drugs used to treat human or animal clinical infections. The market longevity of these disinfectants may be increased by applying only the minimal concentration necessary to mitigate the presence of *Salmonella*. This would have the added benefit of reducing costs to the producer. The continued use of disinfectants that have poor control but have high application rates may function to raise resistance levels in bacteria. Components, such as THN, which are no longer effective, should be removed from formulations where possible.

Acknowledgments

We thank Jackie Kotzur and Kathleen Andrews for technical support. This work was funded by the Agricultural Research Service, U.S. Department of Agriculture. Mention of trade names, proprietary products, or specific equipment is solely for the purpose of providing specific information and does not constitute a guarantee, warranty, or endorsement by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products.

Disclosure Statement

No competing financial interests exist.

References

Aarestrup FM and Hasman H. Susceptibility of different bacterial species isolated from food animals to copper sulphate, zinc chloride and antimicrobial substances used for disinfection. Vet Microbiol 2004;100:83–89.

Anderson PN, Hume ME, Byrd JA, *et al.* Molecular analysis of *Salmonella* serotypes at different stages of commercial turkey processing. Poult Sci 2010;89:2030–2037.

Andrews JM. Determination of minimum inhibitory concentrations. J Antimicrob Chemother 2001;48(Suppl S1):5–16.

Beier RC, Bischoff KM, and Poole TL. Disinfectants (biocides) used in animal production: antimicrobial resistance considerations. In: *Preharvest and Postharvest Food Safety: Contemporary Issues and Future Directions*. Beier RC, Pillai SD, Phillips TD, and Ziprin RL (eds.). Ames, IA: Blackwell Publishing Professional, 2004, pp. 227–238.

Beier RC, Bischoff KM, Ziprin RL, *et al.* Chlorhexidine susceptibility, virulence factors, and antibiotic resistance of beta-hemolytic *Escherichia coli* isolated from neonatal swine with diarrhea. Bull Environ Contam Toxicol 2005;75:835–844.

Beier RC, Duke SE, Ziprin RL, *et al.* Antibiotic and disinfectant susceptibility profiles of vancomycin-resistant *Enterococcus faecium* (VRE) isolated from community wastewater in Texas. Bull Environ Contam Toxicol 2008;80:188–194.

Block SS. *Disinfection, Sterilization, and Preservation*, 5th edition. Philadelphia, PA: Lippincott Williams and Wilkins, 2001, p. 325.

Braoudaki M and Hilton AC. Adaptive resistance to biocides in *Salmonella enterica* and *Escherichia coli* O157 and cross-resistance to antimicrobial agents. J Clin Microbiol 2004;42:73–78.

Byrd JA, McElroy AP, Stevens SM, *et al.* Microbial intervention strategies for Campylobacter reduction during commercial turkey processing in the United States. Turkeys 2006;54:17–22.

Caldwell DJ, Byrd JA, and McElroy AP. National Turkey Federation immersion chiller best management practices (BMP): optimization of microbial intervention strategies. NTF Technical Report. 2003, p. 23.

Capita R, Álvarez-Astorga M, Alonso-Calleja C, *et al.* Occurrence of salmonellae in retail chicken carcasses and their products in Spain. Int J Food Microbiol 2003;81:169–173.

[CDC] Centers for Disease Control and Prevention. Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food—10 States, 2006. Morb Mortal Wkly Rep 2007;56:336–339.

[CLSI] Clinical and Laboratory Standards Institute. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically. Approved standard, 7th edition. M7-A7.* Wayne, PA: Clinical and Laboratory Standards Institute, 2006.

[CLSI] Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Susceptibility Testing, fifteenth Informational Supplement, M100-S15.* Wayne, PA: Clinical and Laboratory Standards Institute, 2005.

Corry JEL, Allen VM, Hudson WR, *et al.* Sources of salmonella on broiler carcasses during transportation and processing: modes of contamination and methods of control. J Appl Microbiol 2002;92:424–432.

Ferber D. Superbugs on the hoof? Science 2000;288:792–794.

Garbarino JR, Bednar AJ, Rutherford DW, *et al.* Environmental fate of roxarsone in poultry litter. I. Degradation of roxarsone during composting. Environ Sci Technol 2003;37:1509–1514.

George AM. Multidrug resistance in enteric and other Gram-negative bacteria. FEMS Microbiol Lett 1996;139:1–10.

Heath RJ and Rock CO. A triclosan-resistant bacterial enzyme. Nature 2000;406:145–146.

Jørgensen F, Bailey R, Williams S, *et al.* Prevalence and numbers of *Salmonella* and *Campylobacter* spp. on raw, whole chickens in relation to sampling methods. Int J Food Microbiol 2002; 76:151–164.

Joynson JA, Forbes B, and Lambert RJW. Adaptive resistance to benzalkonium chloride, amikacin and tobramycin: the effect on susceptibility to other antimicrobials. J Appl Microbiol 2002;93:96–107.

Lapierre L, San Martin B, Araya-Jordán C, *et al.* Comparison of integron-linked antibiotic resistance genes in strains of *Salmonella* spp. isolated from swine in Chile in 2005 and 2008. Can J Microbiol 2010;56:515–521.

Leelaporn A, Paulsen IT, Tennent JM, *et al.* Multidrug resistance to antiseptics and disinfectants in coagulase-negative staphylococci. J Med Microbiol 1994;40:214–220.

Levy SB. Active efflux, a common mechanism for biocide and antibiotic resistance. J Appl Microbiol Symp Suppl 2002;92: 65S–71S.

Li Y and Mustapha A. Evaluation of four template preparation methods for polymerase chain reaction-based detection of *Salmonella* in ground beef and chicken. Lett Appl Microbiol 2002;35:508–512.

Liljebjelke KA, Hofacre CL, Liu T, *et al.* Vertical and horizontal transmission of *Salmonella* within integrated broiler production system. Foodborne Pathog Dis 2005;2:90–102.

Maris P. Resistance of 700 Gram-negative bacterial strains to antiseptics and antibiotics. Ann Rech Vet 1991;22:11–23.

McDonnell G and Russell AD. Antiseptics and disinfectants: activity, action, and resistance. Clin Microbiol Rev 1999;12: 147–179.

Mead PS, Slutsker L, Dietz V, *et al.* Food-related illness and death in the United States. Emerg Infect Dis 1999;5:607–625.

Mølbak K, Baggesen DL, Aarestrup FM, *et al.* An outbreak of multidrug-resistant, quinolone-resistant *Salmonella enterica* serotype Typhimurium DT104. New England J Med 1999;341: 1420–1425.

Nikaido H. Multidrug efflux pumps of Gram-negative bacteria. J Bacteriol 1996;178:5853–5859.

Russell AD. Introduction of biocides into clinical practice and the impact on antibiotic-resistant bacteria. J Appl Microbiol Symp Suppl 2002;92:121S–135S.

Sapkota AR, Price LB, Silbergeld EK, *et al.* Arsenic resistance in *Campylobacter* spp. isolated from retail poultry products. Appl Environ Microbiol 2006;72:3069–3071.

Sidhu MS, Heir E, Leegaard T, *et al.* Frequency of disinfectant resistance genes and genetic linkage with β-lactamase transposon Tn552 among clinical staphylococci. Antimicrob Agents Chemother 2002a;46:2797–2803.

Sidhu MS, Sørum H, and Holck A. Resistance to quaternary ammonium compounds in food-related bacteria. Microb Drug Resist 2002b;8:393–399.

[USDA-ERS] U.S. Department of Agriculture-Economic Research Service. Foodborne illness cost calculator: all sources of *Salmonella* infections. 2010. Available at http://www.ers.usda.gov/Data/FoodborneIllness/, last updated May 13, 2010; last accessed December 20, 2010. Washington, DC: USDA-Economic Research Service. (Online.)

Vadhanasin S, Bangtrakulnonth A, and Chidkrau T. Critical control points for monitoring salmonellae reduction in Thai commercial frozen broiler processing. J Food Prot 2004;67: 1480–1483.

White DG and McDermott PF. Biocides, drug resistance and microbial evolution. Curr Opin Microbiol 2001;4:313–317.

Address correspondence to:
Ross C. Beier, Ph.D.
Southern Plains Agricultural Research Center
Agricultural Research Service
U.S. Department of Agriculture
College Station, TX 77845

E-mail: ross.beier@yahoo.com